13:39:54 OCA PAD INITIATION - PROJECT HEADER INFORMATION 06/13/90

Active

Project #: G-33-A15 Cost share #: Rev #: 0
Center # : 10/24-6-Q5250-5A0 Center shr #: OCA file #:
Work type : RES
Contract#: 5 R01 EY01746-15 Mod #: Document : GRANT
Prime #: Contract entity: GIT

Subprojects ? : N
Main project #:

Project unit: CHEM Unit code: 02.010.136
Project director(s):
YU N-T CHEM (404)894-4007

Sponsor/division names: DHHS/PHS/NIH / NATL INSTITUTES OF HEALTH
Sponsor/division codes: 108 / 001

Award period: 900501 to 910430 (performance) 910730 (reports)

Sponsor amount	New this change	Total to date
Contract value	216,393.00	216,393.00
Funded	216,393.00	216,393.00
Cost sharing amount		0.00

Does subcontracting plan apply ?: N

Title: COMPARATIVE RAMAN STUDIES OF HUMAN AND ANIMAL LENSES

PROJECT ADMINISTRATION DATA

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Security class (U,C,S,TS) : U ONR resident rep. is ACO (Y/N): N
Defense priority rating : N/A NIH supplemental sheet
Equipment title vests with: Sponsor GIT X

Administrative comments -
INITIATION OF PROJECT -15TH YEAR. CONTINUATION OF G-33-A14.

GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION

NOTICE OF PROJECT CLOSEOUT

Closeout Notice Date 08/21/91

Project No. G-33-A15_____ Center No. 10/24-6-Q5250-5A0_

Project Director YU N-T_____ School/Lab CHEMISTRY_____

Sponsor DHHS/PHS/NIH/NATL INSTITUTES OF HEALTH_____

Contract/Grant No. 5 R01 EY01746-15_____ Contract Entity GIT_

Prime Contract No. _____

Title COMPARATIVE RAMAN STUDIES OF HUMAN AND ANIMAL LENSES_____

Effective Completion Date 910430 (Performance) 910730 (Reports)

Closeout Actions Required:	Y/N	Date Submitted
Final Invoice or Copy of Final Invoice	Y	910729
Final Report of Inventions and/or Subcontracts	N	_____
Government Property Inventory & Related Certificate	N	_____
Classified Material Certificate	N	_____
Release and Assignment	N	_____
Other _____	N	_____

CommentsWILL BE BILLED ELCTRONICALLY BY FCTR; CONTINUED BY PROJ G-33-A16._____

Subproject Under Main Project No. _____

Continues Project No. _____

Distribution Required:

Project Director	Y
Administrative Network Representative	Y
GTRI Accounting/Grants and Contracts	Y
Procurement/Supply Services	Y
Research Property Managment	Y
Research Security Services	N
Reports Coordinator (OCA)	Y
GTRC	N
Project File	Y
Other _____	N
_____	N

SECTION IV PROGRESS REPORT SUMMARY	GRANT NUMBER EY01746-16	
PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR Yu, Nai-Teng	PERIOD COVERED BY THIS REPORT FROM 05/01/90	THROUGH 02/20/91
APPLICANT ORGANIZATION Georgia Institute of Technology		

TITLE OF PROJECT *(Repeat title shown in item 1 on first page)*

Comparative Raman Studies of Human and Animal Lenses

(SEE INSTRUCTIONS)

1. The Plans for the Next Year of Support:

The specific aims for the next year of support are : (1) To conclude our FT-Raman studies of human brunescence cataracts with a publishable manuscript; (2) To study fluorescent lipid peroxidized products from Emory mouse lenses and human lenses, as well as model compounds, by the fluorescence-free FT-Raman method; (3) To investigate the nature of protein structural changes associated with H_2O_2 reaction in isolated crystallins and intact lenses by near-IR FT-Raman method; (4) To compare (hence interprete) various fluorescence images of the same human lens excited at different excitation wavelengths (406.7, 457.9, 488.0, 514.5, 568.2 and 647.1 nm); (5) To obtain UV-induced fluorescence distribution profiles of guinea pig lens by long-term *in vivo* UV exposure.

2. Concise Description of the Studies Conducted during the Current Budget Year:

a) *Prevention of Long-Wave UV Induced Damage to the Guinea Pig Lens in vivo by a UV-Blocking Contact Lens*

Previous laser Raman spectroscopic studies of the guinea pig lens showed accelerated -SH to disulfide conversion upon in vivo long-wave UV irradiation (B.C. Barron, N.-T. Yu and J.F.R. Kuck, Jr. Exp. Eye Res. 46, 249 (1988)). Here we report our evaluation of the effectiveness of a UV-blocking contact lens in preventing UV-induced SH oxidation and other associated changes in the guinea pig lens. Guinea pigs were exposed to long-wave UV (1.3 mW/cm^2, spectral distribution 305-410 nm, max. intensity at 353 nm) for 24 hours per day for up to 16 months. The left eyes were fitted with a UV-blocking contact lens (0% transmittance at wavelengths less than 355 nm) and the right eyes were fitted with a regular contact lens. The SH and fluorescence profiles for each lens were examined at distinct points along the visual axis using laser Raman spectroscopy. The UV-protected and UV-exposed lenses differed in the following ways: (a) a gradual decrease in -SH was observed in the UV-exposed (right) lens versus the UV-protected (left) lens until at 12 months of irradiation, a 20%

difference existed between the left and right lenses; (b) after 16 months irradiation, opacification and intense yellowing of the right lens were observed while the left lens remained clear and colorless; (c) concomitant with lens coloration, an unidentified, photochemically produced fluorophor was detected in the right lens (ex./em. 514.5/580.4) yet the left lens exhibited no such fluorescence. The above data indicates that the UV-blocking contact lens utilized in this study provided significant protection from lens damage resulting from continuous, long-wave UV exposure in vivo. In consideration of normal aging studies which have shown similarities between human and guinea pig lenses (N.-T. Yu, D.C. DeNagel, P.L. Pruett and J.F.R. Kuck, Jr. Pro. Natl. Acad. Sci. USA, 82, 7965 (1985)), the present study takes on special significance in terms of the potential for protection from UV-induced changes which may be associated with aging and cataractogenic processes in the human lens.

b) *Non-Invasive Assessment of Human Lens Aging and Cataract Formation by Near-Infrared FT-Raman*

Lens crystallin structural changes are closely associated with lens aging and cataractogenesis. Our progress toward a better understanding of these changes, however, has been hampered by the lack of a suitable non-invasive technique that can provide detailed molecular-level information on intact ocular lenses under natural physiological conditions. Near-IR Fourier transform (FT)-Raman spectroscopy is such a technique. The specific questions addressed are the concentration of disulfide and tryptophan and the conformation of the protein backbone. These parameters are of particular interest because of the hypothesized involvement of SH and tryptophan in cataractogenesis and the speculation that cataract formation may be initiated by a conformational change.

By non-invasively examining a large number (statistically significant) of intact normal and cataractous human lenses, we have provided some *definite answers* to the above questions. For non-cataractous lenses, the conversion of S-H to S-S is rather slow and it becomes significant only after the 7th or 8th decade; in one particular case involving a 90-year-old normal human lens, the detection of intense FT-Raman signal from S-H moieties indicate that the sulfhydryl groups are capable of maintaining in their reduced state even at such an old age. For senile cataractous and brunescent lenses, no S-H Raman signals could be observed, indicating the elevated accumulation of disulfide. Protein conformational changes appear however to be minor both in old human lenses

and in cataractous and brunescent human lenses. A slight decrease of tryptophan concentration (relative to that of phenylalanine) is detected in the highly pigmented nuclei of cataractous human lenses. Finally, the non-invasive nature and high information content of near-infrared-excited FT-Raman may also render it useful for clinical examinations of lens aging and cataract development in patients.

(c) *Near-Infrared FT-Raman in Laser Corneal Surgery: A Noninvasive and Fingerprinting Diagnostic Technique*

The recently developed technique of near-infrared Fourier transform (FT)-Raman spectroscopy has been applied for the first time to characterize normal / pathological human corneas as well as synthetic biomaterials that can be attached to the corneal surface for laser refractive surgery. Unlike most other optical diagnostic and control methods such as optical absorption and fluorescence, laser-excited FT-Raman is intrinsically a fingerprinting technique and is capable of providing detailed information on the structure and composition of normal and pathological tissues. We demonstrate that the near-IR-excited FT-Raman technique is particularly well-suited for noninvasive analysis of intact biomedical samples because it exhibits such attractive features as complete fluorescence elimination, great sampling flexibility, high data acquisition speed and measurement accuracy. High-quality FT-Raman spectra have successfully been obtained for the whole human cornea and the distinguishable layers including the epithelium layer, the Bowman's layer, the stroma and Descemet's membrane. The molecular composition was measured in 5 intact normal corneas as well as in the surgically separated layers. In two keratoconus corneas, unique Raman peaks around 550 cm^{-1} were apparent. Our initial FT-Raman study of UV-crosslinked collagen biomaterials for adjustable synthetic epikeratoplasty also yielded valuable information; for example, the FT-Raman measurement revealed that the collagen and water contents were strikingly comparable in the human cornea and in the UV-crosslinked (20 minutes) collagen. The detailed composition of the synthetic material appeared to be considerably different from that of the stroma but more similar to that of the Bowman's layer, as judged by their distinctive spectral features at 397, 541 and 1095 cm^{-1}. Such molecular information is important to the development of a laser corneal surgical procedure that may achieve widespread clinical and public acceptance.

(d) *Resonance Raman Detection of a Carotenoid in the Lens of the Deep-Sea Hatchetfish*

A laser scanning microprobe has been used to elicit resonance Raman signals from sections of the lens of the deep-sea hatchetfish, *Argyropelecus affinis*. The signals demonstrate with certainty the presence of a carotenoid and its distribution in the lens. The carotenoid exhibits characteristic resonance Raman vibrational modes at 1551 cm^{-1} (C=C stretch, ν_1), 1147 cm^{-1} (C-C stretch with C-H bend, ν_2), 2285 cm^{-1} ($2\nu_2$) and 2681 cm^{-1} ($\nu_1+\nu_2$), upon excitation at 441.6 nm. Unlike glycogen in the nucleus of dove lens, the carotenoid in the *A. affinis* lens occurs at a higher concentration in the cortex, although its presence in the nucleus is established. A study of lenses of varying age shows that carotenoid incorporation is accelerated as the fish grows older and hence its concentration is highest in the cortex. Because of the extremely low concentration of the carotenoid in the nucleus, it was detectable only by the very sensitive resonance Raman technique.

(e) *Monitoring UV-crosslinking of Type I Collagen by Near-Infrared FT-Raman Spectroscopy*

It is possible to polymerize type I collagen gel (Collagen Corp. Palo Alto, CA) under exposure to UV radiation. Potential applications of a photoactivating collagen gel include use as an adhesive for closing corneal wounds, manufacturing and attaching epikeratoplasty lenticules, and use as a lens replacement material for endocapsular surgery. Non-invasive, real-time and in-situ monitoring of the UV collagen crosslinking process is one of the critical problems facing these applications.

Raman scattering characteristics of aqueous type-I collagen following 0, 10, and 20 minute UV crosslinking periods were studied. We also compared a 30-minute crosslinked collagen to the collagen without any UV irradiation. The samples were prepared in a 5"x3"x3" glass chamber filled with moisturized nitrogen. A Pen-Ray UV lamp (UVP, Inc. San Gabriel, CA) with an emission peak at 254 nm was used for the UV source and provided a radiant exposure of 0.73+0.002 mW/cm^2. All FT-Raman spectra were obtained at 4.0 cm-1 resolution using a Bruker FRA 106 FT-Raman spectrometer. Our results show that the Raman scattering intensity ratios between the spectrum peak associated with C-H bond (at 2950 cm-1) and O-H bond (at 3200 cm-1) are 0.33, 0.76 and 1.20 for 0, 10 and 20 minute crosslinking time respectively, representing increased stability of the collagen fiber through the enhancement of hydrophobic bonds (C-H) due to exclusion of "organized water" in soluble collagen during the

crosslinking process. Similar spectral features between the collagens with and without UV crosslinking indicate the absence of chemical composition changes induced by the UV radiation.

Our study demonstrated that near-IR FT-Raman spectroscopy can be used as a real time, quantitative measure of collagen crosslinking for ophthalmic applications.

3. No change

4. Not Applicable

5. **Publications:**

(i) Nie, S., Castillo, C. G., Bergbauer, K. L., Kuck, J. F. R., Jr., Nabiev, I. R. and Yu, N.-T. (1990) "Surface-Enhanced Raman Spectra of Eye Lens Pigments" Appl. Spectrosc., *44*, 571-575.

(ii) Yu, N.-T., Bando, M. and Kuck, J. F. R., Jr. (1990) "Localization of UV-induced Changes in Mouse Lens", Exp. Eye Res., *50*, 327-329.

(iii) Nie, S., Bergbauer, K. L., Ho, J. J., Kuck, J. F. R., Jr. (1990) "Applications of Near-Infrared Fourier Transform Raman Spectroscopy in Biology and Medicine", Spectroscopy, *5*, 24-32.

(iv) Zigman, S., Paxhia, T., Lou, M. and Yu, N.-T. (1990) "Comparative Study of Lens Proteins of Gray Squirrel and Human", Comp. Biochem. Physiol. *96*B, 697-704.

(v) Nie, S., Bergbauer, K. L., Kuck, J. F. R., Jr. and Yu, N.-T. (1990) "Near Infrared Fourier Transform Raman Spectroscopy in Human Lens Research" Exp. Eye Res., 51, 619-623.

(vi) Yu, N.-T., Cai, M.-Z., Lee, B. S., Kuck, J. F. R., Jr., McFall-Ngai, M., and Horwitz, J. (1991) "Resonance Raman Detection of a Cartenoid in the Lens of the Deep-Sea Hatchetfish", Exp. Eye Res. (in press).

(vii) Chen, W., Nie, S., Kuck, J. F. R., Jr. and Yu, N.-T. (1991) "Near-IR Fourier Transform Raman and Conventional Raman Studies of Calf γ-Crystallins in the Lyophilized State and in Solution", Biophys. J. (submitted).